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PROGRESS REPORT

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HUMAN NUTRITION RESEARCH DIVISION MARKETING AND NUTRITION RESEARCH

PROCUREMENT SECTION
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July 1, 1970

Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

This progress report includes a summary of the current research of the Division and a preliminary report of progress made during the preceding 15 months. The summaries of progress include some tentative results that have not been tested sufficiently to justify general release. Because of this, the report is not intended for publication. Copies are distributed only to members of the Division staff and others having a special interest in the development of the Division's research programs.

The report was compiled in the Human Nutrition Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland.

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Issued March 1971

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PROGRESS REPORT OF THE HUMAN NUTRITION RESEARCH DIVISION,
MARKETING AND NUTRITION RESEARCH, July 1, 1971

INTRODUCTION

The research reported here presents recent progress in understanding the nutritional needs of normal man and the manner by which these needs can best be met by food. The research involves studies of the absorption, transport, and metabolism of individual nutrients in the body as related to age, activity, heredity, and environmental conditions. Studies of metabolic processes and nutritional requirements in man are preceded, guided, and expedited by results from intensive studies on laboratory animals and lower forms of life in which more factors can be controlled and physiological responses can be measured during each stage in the life cycle and during successive generations. The research includes the nutritive values of foods as measured by chemical or physical means and by biologic response, and the effects of household practices upon the nutritive value of foods. Investigations are made also of the effect of pesticide use upon human nutrition, food needs and uses. Knowledge gained from human nutrition research can be used to influence the food habits and improve the nutritional status of man. It also can influence market demand and in turn the production of agricultural products.

The program is carried on by the Human Nutrition Division of the Agricultural Research Service of the U. S. Department of Agriculture. It is conducted at the Agricultural Research Center near Beltsville, Maryland, and under contract and cooperative agreement and grants with universities, industry, and private research laboratories. In addition, the Division collaborates with regional programs of the State experiment stations. A field laboratory was constructed at Grand Forks, North Dakota, during Fiscal Year 1970. The major research focus will be on trace mineral requirements. The new facility also provides a 9-bed metabolic unit for controlled diet studies with human subjects. The Federal Scientific effort devoted to this research in Fiscal Year 1970 totalled about 37.3 scientific man-years with 33.9 engaged in the program near Beltsville, Maryland, and the equivalent of 3.4 in contract, grant and cooperative agreements.

Basic information on human nutrition is needed for conservation and optimal utilization of human and food resources and to promote the nutritional well-being, health, and personal satisfactions of the population. The Division has contributed to this goal by providing information on nutritional and food needs to consumers. Some of these contributions have been summarized here:

Gaps in knowledge of human requirements. The need for more research focused on the nutritional needs of adolescents and the elderly is shown by ARS reviews of the world literature on the requirements of man for protein and amino acids. The reviews reveal no reports of research during the past 50 years on the needs of healthy adolescents for these nutrients. For this reason, current protein recommendations for teenagers are extrapolations from results of studies with adults and younger children. Next to teenagers, studies of the protein and amino acid requirements of the elderly have been most neglected. Among the elderly, relatively few individuals in each age group have been studied and the results lack agreement. The reviews on protein and amino acids are the first in a series on nutrient requirements to be carried out by ARS nutritionists in cooperation with the Harvard School of Public Health. The information provided will help to set priorities in planning and evaluating research programs in human nutrition.

Constituents of saliva used to measure response to diet. Levels of some components of saliva are affected by dietary carbohydrate in much the same way that levels of the same components in blood are affected. Preliminary experiments by ARS scientists show an increase in the level of pyruvate, one of the metabolites of carbohydrate, in parotid saliva following an increased intake of either glucose or fructose. Studies are underway to find if other carbohydrate metabolites also are influenced by the kind and amount of carbohydrate. Prior to these studies, normal fasting baseline levels determined for six adult subjects indicated that concentrations of certain constituents of parotid saliva are sufficiently consistent to be used in determining nutritional response. Parotid saliva can be collected with little stress on the subject. The use of saliva instead of blood samples will simplify sample taking and permit more subjects to be included in studies of how the body uses different kinds and different levels of carbohydrates.

Nickel may be an essential nutrient. ARS nutritionists have obtained evidence that nickel may be a nutrient that is required in the diets of animals and humans for optimum health and performance. Recognition that nickel and other trace elements are essential diet constituents is of increasing importance as foods become more refined and formulated. This first evidence of the essentiality and physiological role of nickel was obtained from studies using chickens as the test animal. Chickens are used in these studies because their rapid growth adds to nutritional stresses and makes it possible to demonstrate nutritional deficiencies more quickly than with some other animals. Chicks that received less than 0.08 parts per million nickel in the feed showed physiological changes that were not apparent in chicks that received 3 parts per million. This was evidenced in a dermatitis of the skin on their legs, a slight swelling of the hocks, a bright orange rather than a pale yellow color on the legs, and a reduction in fat content of the liver.

Selenium content of foods. Data on the selenium content of foods from recent research by ARS scientists fill an important gap in our knowledge of the mineral elements in the American diet. Selenium prevents certain muscle and liver diseases in animals and a deficiency of this mineral has been suggested as a complicating factor in human protein-calorie malnutrition. Because selenium occurs in foods in such small amounts, an analytical procedure which measures selenium in the parts per billion (ppb) range had to be used. Seafoods generally contained the largest quantities of selenium, with an average of 532 ppb. Various grain products and meats were the next best sources, with averages of 387 and 224 ppb, respectively. Fruits and vegetables were relatively poor sources of selenium; they contained averages of only 5 and 10 ppb. Further research is needed to evaluate the nutritional value of the different chemical forms of selenium in foods and the possible effects of cooking or processing on the selenium content of foods.

Cooking and curing have little or no effect on the fatty acids in beef and pork cuts. Recently completed ARS contract research has provided essential information on the fatty acid content of raw and cooked beef cuts and of raw, cooked and cured-cooked pork. The work was undertaken to provide data necessary for planning nutritionally adequate meals containing these foods. Information was obtained on the effects of normal cooking methods on meat fatty acids and the effects of curing and cooking on the fatty acids of pork. Individual tissues in each cut were examined to detect any localized changes. The fatty acids were not greatly affected by either cooking or curing, although small changes occurred in some instances. The results provide a justification for the use of data on raw meat in calculating the dietary contribution of cooked or cured meat as eaten. The data will be made available for incorporation into tables of nutrient composition of foods.

Utilization of vitamin A from food sources evaluated. Nutrition scientists in Poland have found that the utilization of vitamin A, especially when supplied by beta-carotene, may be significantly influenced by the other dietary ingredients accompanying the foods that serve as sources of vitamin A. Nutrition studies in the United States as well as in other countries had provided evidence that vitamin A is one of the vitamins frequently found to be in short supply in current diets. Thus, the new finds can help in problems that arise in providing diets containing adequate levels of vitamin A. When the test diet contained casein, gluten, or egg protein, the highest recovery of vitamin A from beta-carotene occurred with egg protein. Protein quality did not seem to provide

an answer to the differences observed. When different vegetable oils and hydrogenated fats were fed, no differences in recovery of beta-carotene as vitamin A were observed, regardless of the source of fat. Both absorption and liver storage (utilization) may be influenced by the kind of dietary carbohydrate. When the only source of carbohydrate in the diet was glucose, wheat starch, or a mixture of wheat starch and glucose, utilization of absorbed carotene or vitamin A was lowest with the glucose-containing diets and highest with the diet containing a mixture of wheat starch and glucose. When wheat white bread, potatoes, or rice flakes served as the source of dietary carbohydrate, absorption of beta-carotene from raw carrots was lowest when the diet contained wheat bread. Under these conditions, however, utilization of the absorbed carotene was not appreciably influenced by the source of dietary carbohydrate. This study was conducted as part of the PL 480 research program.

BENEFITS FROM HUMAN NUTRITION RESEARCH

The Division is participating in an evaluation of human nutrition research in the U.S. The study was undertaken at the direction of the Agriculture Research Policy Advisory Committee of the United States Department of Agriculture. A report "Benefits from Human Nutrition Research" has been prepared for publication as one of a series of reports. Some highlights from the report on benefits follow.

Better health, a longer active lifespan, and greater satisfaction from work, family and leisure time are among the benefits to be obtained from improved diets and nutrition. Advances in nutrition knowledge and its application during recent decades have played a major role in reducing the number of infant and maternal deaths, deaths from infectious diseases, particularly among children, and in extending the productive lifespan and life expectancy. Significant benefits are possible both from new knowledge of nutrient and food needs and from more complete application of existing knowledge. The report "Benefits from Human Nutrition Research" identifies potential benefits from alleviating nutrition related health problems; from increased individual performance and satisfactions; and increased efficiency in food services. A vast reservoir of health and economic assets could be made available by research yet to be done on human nutrition.

Major health problems are diet related. Most all of the health problems underlying the leading causes of death in the United States could be modified by improvements in diet. The leading cause of death, heart and vasculatory disease, has been shown to be associated with changed patterns of fat metabolism. Diet has been implicated in the increased incidence of this condition because fat metabolism is known to respond to dietary change. The relationship of diet to this health problem and others is discussed in greater detail in report No. 2. Death rates for many of the conditions are higher in the United States than in other countries of comparable economic development.

The real potential from improved diet is preventive. Existing evidence is inadequate for estimating potential benefits from improved diets in the terms of health. Most nutritionists and clinicians feel that the real potential from improved diet is preventative in that it may defer or modify the development of a disease state so that a clinical condition does not develop. The major research thrust, nationwide, has been on the role of diet in treating health problems after they have developed. This approach has had limited success. USDA research emphasis has been placed on food needs of normal, healthy persons and findings from this work have contributed much of the existing knowledge on their dietary requirements.

Benefits would be shared by all. Benefits from better nutrition, made possible by improved diets, would be available to the entire population. Each age, sex, ethnic, economic, and geographic segment would be benefited. The lower economic and nonwhite population groups would benefit most from effective application of current knowledge. These savings are only a small part of what might be accomplished for the entire population from research yet to be done. Some of the improvements can be expressed as dollar benefits to individuals or to the Nation. The social and personal benefits are harder to quantify and describe. It is difficult to place a dollar figure on the avoidance of pain or the loss of a family member; satisfactions from healthy, emotionally adjusted families; career achievement; and the opportunity to enjoy leisure time.

Major health benefits are long range. Predictions of the extent to which diet may be involved in the development of various health problems have been based on current knowledge of metabolic pathways of nutrients, but primarily of abnormal metabolic pathways developed by persons in advanced stages of disease. There is little understanding of when or why these metabolic changes take place. The human body is a complex and very adaptive mechanism. For most essential metabolic processes alternate pathways exist which can be utilized in response to physiological, diet or other stress. Frequently, a series of adjustments take place and the ultimate result does not become apparent for a long time, even years, when a metabolite such as cholesterol accumulates. Early adjustment of diet could prevent the development of undesirable long-range effects. Minor changes in diet and food habits instituted at an early age might well avoid the need for major changes, difficult to adopt later in life.

Regional differences in diet related problems. The existence of regional differences in the incidence of health problems has been generally recognized and a wide variation in death rates still exists among geographic areas. These differences in death rate may reflect the cumulative effect of chronic low intake levels of some nutrients throughout the lifespan and by successive generations. A number of examples of regional health problems attributable to differences in the nutrient content of food or to dietary pattern could be given. Perhaps the best known is "the goiter belt" where soils and plants were low in iodine and the high incidence and death rate for goiter was reduced when the diet was supplemented with iodine. Another situation existed in some of the Southern States where pellagra was a scourge a few decades ago. Corn was the major food protein source for low income families in these areas. The resulting niacin deficiency raised the incidence of pellagra to epidemic proportions.

Migration from the high death rate areas almost always results in a reduction in the death rate, although the improvement never approaches the level achieved by those who were born and continued to live in the low rate areas. Similarly, persons who move from low rate areas into higher areas lose part of the advantage. If the death rate for one of the high death rate areas, Wilkes Barre, Pennsylvania, were applied to the entire U.S. population, 140,489 more persons under 65 years would have died per year. If the death rate for one of the lower rate areas, Nebraska, had prevailed, there would have been 131,634 fewer deaths. The highest death rate areas generally correspond to those where agriculturists have recognized the soil as being depleted for several years. This suggests a possible relationship between submarginal diets and health of succeeding generations.

HUMAN REQUIREMENTS FOR FATS AND FOODS TO MEET THESE NEEDS

Problems and Objectives

Many major health problems in the United States are associated with undesirable patterns of fat metabolism. Among these health problems are coronary and vascular diseases and obesity. Fat metabolism is known to respond to changes in dietary fat. However, the desirable patterns of fat metabolism, the manner in which diet influences them, and the implications for physiological response and health are not well understood. Among urgent problems in research on human nutrition and dietary needs, high priority is given to determining the optimal amount of fat needed in the diet and perhaps of even greater importance, the type of fat. There is ample evidence that amount and type of fat are important, but present knowledge is insufficient to arrive at general recommendations for dietary fat. Over the years there have been changes in both the amount and type of fat consumed in U.S. diets. Also, technology advances have brought about changes in fats. The changes have nutritional significance but are inadequately documented.

Nutritional requirements must be expressed in terms of foods and diets if advances in nutrition knowledge are to benefit people. The relationship of dietary fat to heart disease, atherosclerosis, blood coagulation and thrombosis has been suggested by several studies but the cause and effect hypothesis has not been proven. When nutrition research has progressed sufficiently that specific dietary recommendations can be made, it may be possible to modify a sizable percentage of the heart and vasculatory cases and increase the productive lifespan and work efficiency of people. The magnitude of the potential benefits can be appreciated if one notes that some 28 million adults in 1960-1962 were diagnosed or suspected to have heart and vasculatory disease. The economic costs of death from heart disease were calculated at nearly \$32 billion annually.

Major objectives of the research are to develop recommendations for fat intake by humans and to identify the amount, kind, and assortment of foods needed. This will involve:

1. Identifying the individuals or groups of individuals who could benefit from dietary regulation of fat and the age the regulation should begin.
2. Finding out what fat constituents, such as cholesterol and fatty acids in the diet, need to be regulated and how they should be regulated.
3. Determining the amount, composition, and availability of the various lipid components in food.
4. Determining the extent and manner by which non-fat diet constituents may influence recommendations for fat intake.

Progress - USDA and Cooperative Program

A. Behavior of Sterols in Biological Systems

Research was initiated to examine the molecular structure and shape of sterol molecules which determine their activities and linkages with other lipid materials. The intent of the study is to explain why certain sterols such as cholesterol are more readily absorbed in the human system. The work to date has been carried out in model systems. The procedures also will be applied in the study of the tendency of cholesterol to associate with other lipids and plasma for purposes of transport and in the process of deposition. This research shows promise of providing an insight into differences among individuals and their metabolic response to the dietary control of sterols and other lipids. Later integration of the work with metabolic studies in man and animals is anticipated.

B. The Fatty Acid Structure of Triglycerides

Research was initiated on the molecular structure and properties of a number of lipids for the purpose of finding a means for identifying the naturally occurring forms of lipids in foods and in lipid metabolites in the body. A preliminary look was taken at the mass spectra of triglycerides as an approach to a more rapid method for structural analyses of fatty acids in triglycerides. A manuscript entitled "Application of Negative Ion Mass Spectrometry to the Identification of Long Chain Aldehydes and Alcohols" has been accepted for publication in Lipids. Another manuscript is in preparation.

C. Tocopherols in Human Plasma

Tocopherols in the blood plasma of a group of men were determined, using a new method developed for the analysis of specific tocopherol forms in small samples. Only alpha and gamma tocopherols were found. The method was compared with older ones and found to be more specific and to require less sample. Work was initiated to relate human blood tocopherol patterns to the nutrients in a known diet and to other physiological characteristics. A manuscript entitled "Determination of Plasma Tocopherols by Gas Liquid Chromatography" has been accepted for publication by Lipids.

D. Cholesterol Metabolism

Research continued on the effects of dietary fat upon cholesterol metabolism. The absorption, excretion, and synthesis of sterols in rats have been studied using labeled cholesterol. Rats have been fed diets differing in the type and level of dietary fat and level of cholesterol. The absorption, excretion, and synthesis of sterols have been studied using labeled cholesterol. The fatty acids found in cholesterol esters and other lipids from various tissues have been measured. Inbred rats that develop obesity and high plasma cholesterol and lipid levels have been fed diets differing in the level of biotin. Liver biotin, tissue lipids, and plasma insulin are being measured on these rats. The incorporation of $^{14}\text{CO}_2$ into various tissue constituents is also being measured. A number of manuscripts are in process.

E. Predictability of Hypercholesterolemia

A project was initiated in which the response of healthy men of known blood type to different levels of dietary cholesterol was to be observed. In surveys reported recently in the literature, higher serum cholesterol levels were found more among subjects with Lewis (a^+) blood type than among those with Lewis (a^-) type. There also seemed to be an inverse relationship between level of serum cholesterol and activity of intestinally secreted alkaline phosphatase. Preliminary work in our laboratories had shown that in some people under ordinary living conditions, the level of serum cholesterol was quite stable over three consecutive days, whereas in others the cholesterol varied considerably from one day to the next. The current research has been undertaken to study whether blood type, presence or absence of intestinally secreted alkaline phosphatase, and stability of serum cholesterol levels are related to responsiveness of serum cholesterol to dietary stimulus. The findings are expected to assist in predicting which persons could benefit most by modification of dietary intake, and what modifications, if any, should be recommended for different individuals.

F. Rat Substrains Developed With Different Lipid Metabolism Patterns

A number of rat substrains were developed that differed in their lipid metabolic response to diet. The data on heritability of organ size, serum cholesterol level, and nephrosis are being evaluated statistically. A number of manuscripts are in preparation. One manuscript "Rat Strain and Sex Differences in Voluntary Activity: Relation to Some Body and Serum Components" has been accepted for publication in Laboratory Animal Care. One substrain showing different cholesterol and triglyceride responses than the parent strain also has a greater biotin requirement. These findings have been prepared for publication, "Effects of Dietary Biotin on Body Weight and Food Intake, Liver and Serum Composition of Inbred Rats."

G. Dietary Cholesterol and Lipid Metabolism

To better understand the role of heredity in lipid metabolism and response to dietary lipid, we compared the effects of a diet containing egg as a source of protein and lipid on the plasma lipid composition of two strains of rats that differ in their lipid metabolism. The effect of egg in the diet on the plasma cholesterol levels of BHE and Wistar rats was studied after 150 and 350 days of feeding. The plasma cholesterol levels of BHE rats fed the egg-containing diets were greatly elevated; those of the Wistar rats were only slightly elevated. The egg feeding changed the fatty acid composition of the plasma sterol esters of both strains; there was a two- or threefold increase in the 18:1 and a decrease in the 20:4 and 18:2 fatty acids. These differences were found whether the fat in the diet was a blend of fats similar to that in human diets as indicated by the Household Food Consumption Survey of 1955 or a mixture of 14% beef suet and 2% corn oil. At 350 days, there was a slight elevation of the 18:2 fatty acid and sterol esters of the rats on the fat blend diet as compared with the beef suet and corn oil diets. The large amounts of 20:4 fatty acids in the plasma lipid fractions indicates extensive conversion of 18:2 to 20:4 fatty acid in the egg-fed animals.

Differences were seen between the Wistar and BHE rats. The Wistar rats fed the fat blend-egg diet for 150 days had higher 20:4 fatty acid content in the plasma sterol esters than the BHE rats. By 350 days, the plasma sterol esters of the Wistar rats on the blend-egg diet had a higher content of 18:2 and 20:4 and lower content of 18:1 fatty acids than the BHE rats. Similar results were found in the 18:1 and 18:2 fatty acids of the plasma sterol esters in the rats fed the fat blend without added egg.

The fatty acid composition of plasma triglycerides and phospholipids did not respond to dietary fat as strikingly as the sterol esters. The triglycerides of the rats fed the basal diet, 14% beef suet and 2% corn oil but no egg, had lower 18:2 fatty acid content than either the fat blend or fat blend plus egg fed groups. The lower proportion of 18:2 to 20:4 fatty acid in the plasma cholesterol esters of the BHE animal at 350 days as compared to the Wistar rat suggests strain differences.

Publications - USDA and Cooperative Program

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Problems and Objectives

Although the human body contains a large variety of mineral elements, Recommended Dietary Allowances issued by the National Academy of Sciences-National Research Council have been proposed for only five minerals. Prior to 1968, only calcium and iron were included. This reflects the dearth of evidence for both minimum and maximum intake of a variety of essential mineral elements known to be required by man. Even when these requirements are known, they must be translated into terms of food and diets if advances in nutritional knowledge are to benefit people. Benefits from mineral nutrition research may be expected to include improved health, a longer productive life, and fewer work days lost. The research leads suggest that optimal intake of minerals in the diet could lead to stronger and better developed bones and teeth with less likelihood of deterioration as in osteoporosis and tooth decay, a lower incidence of anemia with associated reduced stamina and activity, reduced incidence of goiter, a lowering of the incidence of cardiovascular disorders, and numerous other health benefits that can be translated into economic benefits.

Major objectives of the research are aimed at developing recommendations for mineral intake by humans and identification of the amount, kind, and assortment of foods needed. This includes research to:

1. Identify the individuals or groups of individuals who could benefit by altering or regulating their dietary mineral intake.
2. Identify the minerals which need to be regulated in the diet and how they should be regulated.
3. Determine the amount, composition, and availability of the mineral components of food.

Progress - USDA and Cooperative Program

A. Requirement for "New" Trace Elements

A systematic effort has been made to determine nutritional requirement and biological function for trace elements for which no requirement has been established as yet but which are consistently detected in plants and animals when modern, sensitive methods are applied.

To this end, animals are raised in an all plastic, controlled environment allowing maximal exclusion of metallic contamination. The metal content of dietary components is analyzed, and experimental diets are designed that contain all presently known essential ingredients but are low in the trace element under study.

These efforts have led to the discovery that the trace element nickel has a biological function. Feeding a diet with less than 40 parts per billion nickel to growing chicks resulted in dermatitis, slightly swollen legs and joints and changes in fat metabolism, manifested by reduced fat content of the liver. The fact that inclusion of 3 parts per million of nickel in the diet prevented these changes is strong evidence for a nutritionally essential function of this element. Similar efforts applied to the trace element vanadium resulted in significant deficiency symptoms, preventable by vanadium supplementation. These experiments are still in progress. Manuscripts presenting details of these findings have been prepared.

Studies that led to the identification of chromium as an essential element in human and animal nutrition are being continued. They demonstrate that there exists a stringent requirement for specific chemical complexes that bind chromium. In this organically bound form, chromium exerts a much greater biological activity, is absorbed and transported to a greater degree and is more stable against interference from other dietary ingredients than the simple chromic salts. Evidence for the existence of mild chromium deficiency in part of the population is accumulating, particularly in older subjects. Several human studies by other investigators have indicated that the impaired glucose metabolism of many old people is improved by dietary supplements of trace amounts of chromium.

B. Occurrence and Availability of Mineral Elements in Food

The estimate that approximately 10% of the dietary iron is available for absorption is an insufficient foundation for recommending diets that prevent the development of iron-deficiency anemia. It is known that the availability of iron varies considerably with the dietary source of the element, other dietary ingredients and the nutritional state of the subject. In order to determine the utilization of dietary iron more closely, a standardized assay system in rats was developed that permits comparison of dietary iron sources against a standard iron complex. It was found that the iron in egg yolk is of much inferior availability as compared to the standard, but that ascorbic acid added to the diet greatly increases its utilization. Ferritin-iron was highly available, but the nutritionally more important sources, hemoglobin and myoglobin were only 30-40% as available as the standard.

A related study was initiated under a research grant at Seattle, Washington. The Washington study is concerned with the availability of dietary sources of iron to experimental animals and man, as well as with the influence of

food preparation on the availability. A rat assay system has been developed that measures retention of radiolabeled iron by whole body counting and thus makes it possible to test absorption of iron from a variety of foods labeled with radioactive iron in vivo. Suitable standardization procedures have been developed that allow strict control of within-animal variation. Applicability of results from these experiments to human subjects will be investigated by correlating these with human studies.

The biological availability and effectiveness of chromium depends on the binding of the element in a specific form designated glucose tolerance factor (GTF). In an attempt to isolate and chemically identify this factor, initial extraction procedures have been developed. Fractionation of these extracts has resulted in chemical purification as well as definition of chemical properties of GTF. A number of animal and vegetable sources have been tested for GTF activity.

Various microbial species have been tested in order to develop a microbiological assay for GTF. A gram negative rod species has been isolated that responds to GTF-active preparations with increased growth.

C. Food Sources of Selenium

Foodstuffs common in the American diet have been analyzed for their selenium content. Seafoods, grain products and meats contained the most selenium whereas vegetables and fruits contained the least. The findings suggest that the ingredients of average American diets contained adequate nutritional levels of selenium, although effects of cooking and processing, geographical origin and chemical form of selenium remain to be investigated.

D. Calcium Absorption

A study conducted at the University of Connecticut and supported by an ARS grant is concerned with the dynamics of calcium metabolism. The calcium binding activity of intestinal mucosa was found to increase rapidly following restriction of dietary calcium intake. Methods have been developed to study these changes at the level of nucleic acid synthesis and metabolism, so that an effort can be made to investigate the mechanism by which dietary changes lead to increased calcium absorption.

E. Prevention of Iron Deficiency in Infants

Iron deficiency anemia has been found to be one of the most prevalent disorders of childhood in the United States. It is particularly prevalent among Negro children under two years of age. Iron deficiency can, however, be prevented by dietary means. A project has been initiated, therefore, to study the feasibility of preventing the onset

of iron deficiency anemia during the first two years of life by the feeding of iron fortified cereals, iron fortified milk and/or evaporated milk formulas made with iron fortified corn syrup. The project will be conducted under a grant at Beth Israel Hospital in Boston.

F. Iodine Metabolism and Stores in Iodine Deficient Populations

The increased incidence of goiter in the U.S. as shown by nutritional status surveys increases the significance for the U.S. of the findings from PL 480 research being conducted in New Delhi, India, on iodine metabolism in endemic goiter areas. The thyroids from goats in the endemic goiter area produced more tri-iodothyronine (T_3) than thyroxine (T_4) while the opposite was true in normal glands. The T_3 is a more active form of the thyroid hormone than T_4 . This would allow the gland to produce more hormone activity from less iodine in the deficient state. The thyroglobulin from the deficient goat glands were shown to differ from normal thyroglobulin by electrophoresis. The tertiary structure of the thyroglobulin is believed to influence the formation of T_3 and T_4 . The following paper is in press: Karmarkar, M. G., Kodipillai, N., Deo, M. G. and Ramalingaswami, V. "Adaptation of Thyroid Gland to Iodine Deficiency," Life Sciences. An understanding of the thyroid adaptation to iodine deficiency may explain why many individuals do not develop goiter even with low iodine intake. The differences among T_3 and T_4 production may be used to diagnose iodine deficiency even when goiter is not present.

G. Goitrogenic Substances in Vegetables

Research done with PL 480 funds in Olsztyn, Poland, has provided information on the content in vegetables and milk of certain compounds which interfere with thyroid function causing enlargement of this tissue. Cabbage, radish, cauliflower, broccoli, Pekin cabbage, kale, and brussels sprouts were analyzed for 5-vinyl-1,2 oxazolidine-thione (OZT); isothiocyanates (ITC) and thiocyanates (TG). Broccoli and brussels sprouts were high in OZT. Storage of cabbage resulted in loss of ITC and OZT. Fermentation resulted in loss of ITC and TC but not OZT. Heating with steam at 120 for 60 minutes destroyed 30% of OZT. Rapeseed meal caused greater thyroid enlargement than a mixture of OZT, ITC, and TC equivalent to it. OZT causes an increase in I uptake by thyroid while ITC and TC decreases it. The effects of OZT are not overcome by iodine in the diet. Cows fed rapeseed meal did not have OZT in milk but did have TC. Milk from cows fed rapeseed meal did not produce good quality cheese. Substances in the milk interfere with ripening. Levels of OZT, ITC, and TC in vegetables eaten by humans were not high enough to be an important cause of goiter. Publication is planned.

Research on a goitrogen in soybeans was completed in Sendai, Japan, under a PL 480 contract. Defatted soybean was extracted with acetone and

alcohol. Extracts and residues have been fed to rats as a test of goitrogenic activity. The final report has not been received. Until the nature of the goitrogen is unknown, it is not possible to evaluate the importance of this compound in human goiter.

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Problems and Objectives

Recommended dietary allowances for ten vitamins essential to man have been proposed by the National Academy of Sciences - National Research Council. Four of these (folacin and vitamins B₆, B₁₂, and E) were included for the first time in the last revision in 1968. Dietary allowances recommended are for some vitamins based on incomplete information for a number of the age and sex groups. A number of other vitamins are known to be required but information is too fragmentary to provide a basis for establishing recommendations. Recent information from both dietary surveys and nutritional status studies indicates that inadequate dietary intakes of vitamins may be found at all income levels in the U. S.

Human requirements for vitamins must be expressed in terms of foods and diets if advances in nutritional knowledge are to benefit people. Although the major vitamin deficiency diseases in the U. S. have been reduced to a very low level, less obvious but debilitating aspects of improper nutrition are widespread. For example, folacin deficiency is known to contribute to the widespread anemia being observed in nutritional status surveys. Benefits from nutrition research in this area include improved health, a longer productive life, a greater sense of well-being, and fewer work days lost. Major economic benefits would derive from these gains.

Major objectives of the research focus on the goal of developing recommendations for vitamin intake by humans and identification of the amount, kind, and assortment of foods needed to supply the vitamin needs. This includes the following research aims:

1. Identify the individuals or groups of individuals who could benefit from altering or regulating their dietary vitamin intake.
2. Identify the vitamins which need to be regulated in the diet and how they should be regulated.
3. Determine the amount, composition, and availability of the vitamin components of foods.

Progress - USDA and Cooperative Program

A. Interaction of Vitamin E and Selenium

It has been known that the actions of vitamin E and selenium are closely related. As the first part of a project to exactly define the mode of action and interaction of these factors, the protective effect of vitamin E against excessive levels of selenium was investigated in the rat. High levels of vitamin E in the diet diminished the liver damage caused by selenium, but only when sufficient methionine (0.5%) was present. The vitamin could be replaced by some, but not all antioxidants. Attempts to replace methionine with betaine or cystine were unsuccessful, and a methyl-acceptor, guanidoacetic acid, reversed the beneficial effect of methionine. These findings, together with a reduction of tissue selenium levels by above treatment suggest that vitamin E plays a role in reactions leading to methylation of selenium.

B. Unidentified Growth Factors in Cereal Grains

Addition of certain cereal fractions to diets complete with regard to all known essential nutrients resulted in significant increases of growth in rats. These results suggest the existence of hitherto unrecognized essential dietary factors.

C. Vitamin A

A review of the world's literature of the vitamin A requirement of human subjects was initiated. The resumé is being prepared for publication.

The biological availability of vitamin A activity in dietary sources can only be estimated at the present time. It was therefore investigated how processing (blanching, frozen storage, cooking or canning) of vegetables affected their composition with regard to carotene isomers. A method has been developed to separate and determine the various isomers by chromatography. Vegetables were found to differ widely in amounts and kinds of stereoisomers formed as a result of processing. In some cases, 35% of the well available all-trans carotenes were converted to isomers, resulting in significant decrease of provitamin A content.

A study supported by PL 480 funds was conducted in India on the effects of excessive doses of vitamin A in rats. Changes in phospholipid metabolism were observed as early as 3 hours after dosing. At some later intervals there were increases in tissue lipid, but a decrease of glycerides and cholesterol in kidney, and a decrease of phosphorus-32 incorporation into certain phospholipids. Another project supported by an ARS grant also found detrimental effects in the offspring of rats given excessive doses

D. Dietary Factors in Folate Deficiency

Serum folate activity and urinary excretion of PGA has been studied in healthy humans after oral doses of PGA. The incidence of folate deficiency anemia in young children in Upper Galilee is being estimated. Site of folate absorption in rats is being determined. The research is being done under the PL 480 program at the Hebrew University, Hadassah Medical School in Hadassah, Israel. This preliminary work with healthy humans suggests that folates are readily absorbed and that serum folate activity can be used to reflect folate intake.

E. Vitamin B₁₂ Requirement

Research on food factors affecting vitamin B₁₂ requirements has been carried out at Hadassah Medical School, Jerusalem, Israel, as part of the PL 480 program.

Unheated soybean meal fed to rats appeared to increase the requirement for vitamin B₁₂. The feeding of the raw soybean flour induced metabolic disorders characteristic of vitamin B₁₂ deficiency. Biosynthesis of methionine from homocysteine was not the limiting factor in this apparent vitamin deficiency. The project has been directed toward a clearer understanding of factors which render nutrients unavailable to the consumer.

F. Nutrient Needs of Pregnant Adolescents

The nutritional needs of adolescents have received scant attention in controlled dietary studies. Even less is known about their needs when pregnancy imposes an added stress. The pregnant teenager, however, is becoming an increasingly common occurrence in our society. A study supported by a grant has been initiated, therefore, at the University of Vermont to obtain information on dietary intake and nutritional status of pregnant adolescents as compared to that of pregnant adults. The information obtained in the study is expected to provide guidelines on how well the increases in nutrient allowances recommended for women during pregnancy meet the needs of pregnant girls who may be still growing. Emphasis will be placed on vitamins and minerals.

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HUMAN REQUIREMENTS FOR PROTEIN AND AMINO ACIDS AND FOODS TO MEET THESE NEEDS

Problems and Objectives

Proteins and amino acids are the key components in body and tissue growth and development. There is increasing evidence in the scientific literature that a deficiency of these constituents during certain critical periods in human development can seriously and perhaps irreversibly impair not only physical development but mental development as well. Protein deficiency constitutes one of the major deficiency diseases throughout the world. Nevertheless, there are many gaps in our knowledge of human requirements for protein and amino acids. The defining of requirements for amino acids is made difficult by the complexities of interrelationships with other nutrients and among the amino acids themselves. Nutritional requirements must be expressed in terms of foods and diets before advances in nutritional knowledge can benefit people. Diet planning thus is dependent upon knowledge of the kind, amount and biological availability of proteins and their constituent amino acids in our food supply.

Major objectives of the research are to:

1. Identify the individuals or groups of individuals who could benefit from altering or regulating their dietary intake of protein and amino acids.
2. Identify the protein constituents which need to be regulated in the diet and how they should be regulated.
3. Determine the amount, composition, and biological availability of the protein and amino acid components of foods.

Progress - USDA and Cooperative Program

A. Amino Acid Absorption

Unequivocal evidence of relationships between intestinal uptake of amino acids and type of carbohydrate providing the required source of energy would contribute to understanding of the effects of type of carbohydrate on efficiency of nitrogen utilization by animals and man. Previous investigations with a protozoan have shown that the accumulation and utilization of amino acids can be affected at the cellular level by the type of carbohydrate supplied in the growth media. Results of these studies are summarized in a paper accepted by the Journal of Bacteriology. It was of interest, therefore, to determine if intestinal cells of higher animals respond in comparable ways.

Results, which must be considered preliminary at this time, indicate that the uptake of amino acids by rat intestinal tissue in vitro can be altered by varying the type of carbohydrate simultaneously present.

When segments of rat intestinal tissue were incubated in a buffered solution of hydrolyzed casein containing carbohydrate as glucose, dextrin, or glucose plus dextrin, the total uptake of individual amino acids was greater from the medium containing carbohydrate only as glucose than from that with dextrin, with or without the simultaneous presence of glucose. The pattern of absorbed amino acids was not affected by type of carbohydrate in incubation media when the intestinal tissue was from rats on a diet providing carbohydrate only as starch. When the intestinal tissue was from rats on a diet providing carbohydrate only as sucrose, the patterns of amino acids absorbed from media containing dextrin were similar but differed from the pattern observed when the medium contained only glucose.

B. Collagen Metabolism

Investigations into the chemical-physical characteristics of collagen from striated muscle may provide clues to fundamental changes which occur during aging, malnutrition, rehabilitation, and episodes of acute nutritional deprivation. An improved method for the isolation of intramuscular connective tissue (IMCT) has been developed, greatly increasing the yield of this tissue from striated muscles and allowing chemical-physical studies to be extended to the IMCT of cardiac muscle. Variations in inter- and intra-molecular cross-linking, hydrothermal shrinkage characteristics and amino acid composition have been observed in collagen isolated from bovine, ovine and porcine striated muscles, supporting earlier observations that cross-linking and/or synthesis characteristics of IMCT collagen vary markedly among species. Continuation of the studies involving muscle variation has revealed that the amino acid composition and hydrothermal shrinkage characteristics of collagen from active and inactive type striated muscles do not vary significantly, indicating that variations in primary-secondary structure do not account for the marked differences in inter- and intra-molecular cross-linking observed in the IMCT collagen from these two types of muscle. These results are reported in a paper accepted for publication by the International Journal of Biochemistry. Since isolation of collagen requires large amounts of tissue, use of relatively large animals for nutritional studies is being considered, especially since cardiac tissue may be vitally important in assessing changes during aging and nutritional insults. A study of the age associated changes in metabolism of collagen from porcine muscles has therefore been initiated in conjunction with the Animal Husbandry Research Division; in vitro aging changes in collagen cross-linking will also be investigated.

C. Reproduction and Growth on Rice Diets

The problem of growth, reproduction and lifespan during subsistence on low protein rice diets has been the subject of research conducted under contract with WARF. Experiments carried through three generations of rats fed low protein rice diets have demonstrated that although weaning weights were severely depressed, growth and subsequent reproduction could be achieved. Cross lactation studies showed that the depressed

weaning weights were probably caused by failure of rice-fed dams to provide adequate nutrients in the milk. The third generation animals, however, although stunted at weaning, have grown reasonably well and have maintained good health. Supplementation with lysine and threonine improved weaning weights and subsequent growth although these parameters were always substantially lower than in animals fed a high protein casein diet. These experiments are being continued to determine the effect of these low protein diets on the onset of degenerative changes in later life and their effect on lifespan. Systematic investigations of the effects of single grain low protein diets should provide basic knowledge relevant to the nutritional needs of the large numbers of people who now subsist on these diets.

D. Evaluation of Lactose-Containing Milk Products

An investigation of the nutritional value of milk and cheese byproducts which contain large amounts of lactose was initiated. Emphasis is being placed on the capability of these products to provide supplementary protein, calcium, and phosphorous to grain diets containing marginal amounts of these nutrients.

A feeding study was carried out in which normal weanling rats were given rice-based or wheat-based diets supplemented with (1) nonfat dried milk, (2) dried whey, (3) dried whey prepared by a reverse osmosis procedure that removed some lactose, some salts, and some nonprotein nitrogen compounds (R-O whey), (4) soy-whey, a product containing two-thirds whey and one-third full fat soy flour, and (5) soy-whey with part of the lactose hydrolyzed. Total protein in the diets varied from 8.5% (rice-based) to 10% (wheat-based).

Preliminary results showed that, as expected, addition of milk products to wheat and rice based diets improved gains and protein efficiency ratios (PER). When the supplement was whey, rats had diarrhea during the entire feeding period and enlarged ceca at autopsy; nevertheless, weight gains and PERs were equivalent to those of rats fed soy-whey. Weight gains and PERs of rats fed R-O whey compared favorably with those of rats fed nonfat dried milk and were much greater than with dried whey or soy-whey. Calcium and phosphorous utilization data are being evaluated.

In addition to these animal studies, a grant with the Johns Hopkins University has recently been initiated to study the intolerance to lactose among school children of different racial backgrounds, to determine relative incidence of lactose intolerance and the age at which it becomes an appreciable factor influencing the intake of milk in these groups.

E. Effect of Protein Level and Type of Carbohydrate on Aging

An investigation of the interactions between levels of dietary protein and kinds of carbohydrate on growth, reproduction, and aging in rats has recently been initiated. Low, medium and high levels of protein will be fed with sucrose or starch to Wistar and BHE rats. Major criteria of the aging process will be mortality and tissue pathology. Supplementing this research effort is a grant with the Harriet G. Bird Foundation, the purpose of which is to investigate the effect of progressive reduction, with age, of protein intake upon lifespan and tissue pathology in rats.

F. Food Protein and Amino Acid Availability

The nutritional availability of amino acids from food proteins depends on intramolecular chemical reactions which occur during cooking; on intermolecular reactions of active residues with other nutrients; and on the presence of active intestinal enzymes for protein degradation. Studies of the amino acid composition and structural conformation of purified meat fibrillar protein (tropomyosin) have continued. Chemical definition of carbohydrate-amino acid interactions is being carried out to determine possible involvement of amino acid residues in the formation, during cooking, of compounds which may prevent the release of essential amino acids in the intestine and thus render them unavailable to the organism. A cooperative agreement with Columbia University has recently been initiated to determine changes in the biological availability of essential amino acids which may occur as a result of various methods of preparing "convenience" foods. An investigation also has been initiated to examine a heat stable trypsin inhibitor from peanut meal. Characterization of this substance is important in determining the extent to which inhibition of trypsin may affect the availability of essential amino acids from peanuts and also amino acids from other foods in the diet.

G. Protein Value of Tempeh

Protein Efficiency Ratios (PER) of freshly prepared or stored tempeh and that of unfermented soybeans were not significantly different by rat feeding tests at 10% protein level in the diet. Supplementation with lysine, methionine, and threonine to the tempeh diet improved PER to the level of that of the tempeh-whole egg containing diet. This research was done in Osaka, Japan, under the PL 480 research program.

H. Dietary Protein and Learning Performance

PL 480 research at Baroda, India, has provided much information basic to understanding protein malnutrition with regard to possible effect on mental development. Two types of diets were fed, a simple diet with content of individual nutrients varied and one containing locally consumed foods with and without supplements of critical nutrients. Effect of protein deficiency varied with age at which deficiency occurred. During post weaning, psychological performance was poor and enzymes in

the brain concerned with glutamic acid and carbohydrate metabolism were reduced when diet contained 5% protein in contrast to 20% protein. Protein deficiency in maternal diet was without influence when post-weaning diet was adequate. Restricted feeding of a qualitatively adequate diet during post weaning had no effect on brain enzymes. With a high protein diet deficient in vitamin A behavioural response and brain enzymes resembled results with low protein diet. Maze performance and activity of brain enzymes were comparable to results with 10% casein when the poor Gujarati diet was supplemented with locally available legumes and greens so as to increase its content of protein, vitamin A, and riboflavin. Additional publications are in preparation.

I. Tryptophan Requirements

Relationships between plasma tryptophan levels and dietary intakes of young men were studied recently under a grant at Massachusetts Institute of Technology. The results, reported at the FASEB meetings in April 1970, showed that at dietary tryptophan intakes below 3 and above 5 mg/kg bodyweight, plasma tryptophan levels did not respond to dietary changes. As the dietary intake increased from 3 to 5 mg/kg, however, plasma tryptophan increased linearly. The MIT research team interpreted these data as suggesting a tryptophan requirement of 3 mg/kg bodyweight. Nitrogen balance data obtained in the same study indicated a mean minimum requirement of 2.0 to 2.6 mg tryptophan/kg bodyweight. The balance data did not allow for losses through skin, sweat, and hair. Similar studies on serum lysine response did not show as obvious a relationship between intake and serum level as the tryptophan studies did. Studies with valine have not yet been completed.

J. Protein Nutritional Status

There is no satisfactory index for rapidly assessing the adequacy of protein intake that is suitable for use in surveys of populations where mild or moderate protein-calorie malnutrition may exist. A study supported by a grant, was initiated, therefore, at the University of California at Berkeley. The purpose of this research is to obtain information on hair root morphology as an index of protein nutritional status. Preliminary findings indicate that complete protein deprivation is reflected in changes in hair root morphology within 11-14 days.

K. Quality of Protein in Children's Diets

The southern region nutrition research project S-64 on nutritional interrelationships of minerals and other nutrients has afforded us the opportunity of studying the effects of protein quality on various indices of nutritional status. The project, to be carried out under a cooperative agreement with Virginia Polytechnic Institute, will study the response of preadolescent girls to low protein diets with and without added lysine, methionine, and threonine, when two levels of calcium are fed.

Nitrogen balance, urinary creatinine and hydroxyproline, fecal and blood lipids, hemoglobin, hematocrit, height, and weight changes will be observed.

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HUMAN REQUIREMENTS FOR CARBOHYDRATES AND FOODS TO MEET THESE NEEDS

Problems and Objectives

In recent years nutritionists have moved away from the concept of the carbohydrates being essentially calorie-providers, with the different types of carbohydrates being similar in most nutritional respects. This narrow viewpoint failed to indicate any urgency for studies on the various carbohydrate components of foods. The result is that much of our information on carbohydrate content of commodities is a residual figure of carbohydrate "by difference." It is now known that many species and many strains of animals as well as individual human beings show quite different metabolic responses to different dietary carbohydrates. These studies suggest that some of the benefits of carbohydrate nutrition research may relate to heart and vasculatory diseases, which were diagnosed or suspected in 28 million adults during 1960-62. The economic costs of death from heart disease have been calculated at approximately \$32 billion annually. When nutrition research has progressed sufficiently that specific dietary recommendations can be made, it probably will be possible to modify a portion of the heart and vasculatory cases and increase the productive lifespan and work efficiency of people who may differ in their metabolism of dietary carbohydrates.

Major objectives of the research are to:

1. Identify the individuals or groups of individuals who could benefit from altering or regulating their intake of dietary carbohydrate.
2. Identify the carbohydrates which need to be regulated in the diet and how they should be regulated.
3. Determine the amount, composition, and biological availability of the carbohydrate components of food.

Progress - USDA and Cooperative Program

A. Long-term and Short-term Effects of Dietary Carbohydrate in Rats

ARS nutritionists at Beltsville have found that the kind of carbohydrate consumed in the diet of rats produces both long-term and transient effects on metabolism. In a recent study, male rats from two different strains were fed, from the time of weaning, nutritionally adequate diets which differed only in the kind of carbohydrate (sugar, glucose, or starch) they contained. Animals were studied at 3, 6, and 9 months of age. The two strains showed differences in weight, fatness, and in the

activity of many of their tissue enzymes. The kind of carbohydrate fed was found also to influence body weight and fatness, liver weight, and the activities of several hepatic enzymes, but most of these effects were seen only at one of the three ages studied. However, two effects of diet were observed in both strains at all ages. Liver fat and the hepatic enzyme, glucose-6-phosphate dehydrogenase, which is importantly involved in the biological synthesis of fat, were relatively high when sucrose was fed in place of starch. A manuscript describing these findings has been prepared.

B. Carbohydrate Effects on Growth

Scientists working under a PL 480 grant in Israel have been studying how the kind of carbohydrate eaten (glucose, fructose, sucrose, or corn starch) may affect growth and nitrogen utilization. In a recent study, young male rats were fed controlled amounts of diets which differed only in the kind of carbohydrate which each contained. It was found in short-term studies that, whether the diet contained 11 or 18% protein, the starch-fed rats did not retain more nitrogen than did rats receiving equal caloric and protein intakes of the sucrose, glucose, or fructose diets. On long-term feeding of the diets containing 11% protein, however, starch-fed animals gained more weight than did the other groups. It was concluded that the level of protein intake is important in determining whether the kind of carbohydrate consumed affects growth rate.

C. A Role of RNA in Fat Synthesis

ARS scientists at Beltsville have been directing much effort toward understanding how the synthesis of fatty acids from carbohydrate in the liver is regulated. They believe that tendencies toward obesity and high blood lipids in man usually represent an overabundance of certain enzymes that catalyze key reactions in fat synthesis. They are particularly concerned about the role of messenger RNA, large molecules which are directly involved in the synthesis of enzymes and other proteins in cells. Evidence has been obtained in rats that a marked elevation of two key enzymes in the pathway of fat synthesis requires the production of extra messenger RNA. This extra RNA appears to persist during subsequent food deprivation and to influence subsequent dietary responses. Manuscripts detailing the evidence are being prepared.

D. Human Studies with Saliva

Scientists at Beltsville have initiated research on how people differ in their short-term metabolic responses to meals of selected carbohydrates. The main parameters being studied are enzyme and metabolite levels in blood serum and in parotid saliva. A standardized method of collecting saliva from the parotid gland has been developed. Early findings with fasting subjects show large individual differences in

the level of various enzymes and metabolites in parotid saliva. A manuscript incorporating these data has been prepared.

E. Short-term Response to Dietary Carbohydrates

Research, previously in 1966 and 1967 reports, showed that in healthy young men and women the activity of certain enzymes, lactate dehydrogenase (LDH) in particular, were significantly greater when sucrose rather than starch provided most of the dietary carbohydrate. The enzyme activity levels were studied after the subjects had eaten the diets for 30-day periods. Subsequently, a grant was initiated at the University of Alabama, in part to find out whether the LDH response was an immediate reaction to the type of carbohydrate. The results of this research were reported at the FASEB meetings in April 1970. They showed that total LDH activity and LDH isozyme activities in blood drawn at hourly intervals for five hours after a test meal were not significantly different when corn starch, wheat starch, sucrose, glucose, or fructose provided the carbohydrate in the test meal. The response observed in the earlier studies, therefore, cannot be assumed to have been an immediate reaction. The time required for its development, how long it persists after a change in dietary carbohydrate, and whether it has any impact on the health status of the subject are not known. The Alabama research also showed that pyruvate levels were higher after fructose or sucrose had been eaten than after glucose or starch. This observation lends support to the reported findings by this Division in 1967 that suggested different metabolic pathways for sucrose and starch.

F. Carbohydrate-induced Hyperlipemia in Man

In patients with carbohydrate-induced hypertriglyceridemia, serum triglycerides and serum total cholesterol increased during prolonged feeding of sucrose, fructose, and glucose, while decreases were observed during feeding of starch. Normal subjects responded similarly but with less extreme changes. This research was carried out in Israel and was supported by PL 480 funds. In subsequent studies, the fructose moiety of sucrose was implicated as the most potent inducer of this effect, although some increases were observed with glucose feeding. Similar responses were found when sucrose, fructose, and glucose were fed for 1-2 days. There was no difference in the absorption of fructose between subjects responsive and not responsive to fructose. Strong evidence was found of a defect in serum-triglyceride clearance in the patients with carbohydrate-responsive hypertriglyceridemia. In other experiments, with rats, it was found that incorporation of fructose carbohydrate into serum and liver lipid was greater than that of glucose carbohydrate. Adipose lipoprotein lipase activity increased after glucose feeding but not after fructose feeding, which may provide a reason for the lack of effect of glucose on serum triglycerides in the rat. In humans, lipoprotein lipase activity was not affected by administration of glucose.

Although high consumption of saturated fats has been most commonly held responsible for elevated fat in the serum, this research shows that refined sugar (sucrose) may also be responsible for this effect. The widespread and increasing rise of refined sugar in foods thus could be a factor in the etiology of arteriosclerosis. These results indicate the need for more clinical research on the dietary role of sugars in the synthesis and clearance of fat in human subjects.

Another study initiated at the same institution is expected to yield information on the response of coronary-artery disease (CAD) prone people and non-CAD prone people to different dietary carbohydrates. The information will assist in making recommendations on carbohydrate requirements of people of different familial background.

Publications - USDA and Cooperative Program

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EFFECT OF PESTICIDE USE ON HUMAN DIETARY REQUIREMENTS

Problems and Objectives

The pesticide residues found in foods in market basket surveys of foods eaten by the consumer have been below that allowed by Federal regulation. A further reduction in pesticide content is expected in the near future due to the recent ban on use of persistent types of pesticides on certain classes of food crops. As long as this reduction in the use of persistent pesticides within the environment continues, it is felt that this research area has lower priority than some other nutrition problems. Under the circumstances, effort in this area is gradually being transferred to a higher priority research problem area - "Human Nutritional Well-Being." No new projects on pesticide related nutrition problems were initiated during the reporting period. What appears below is progress on projects initiated earlier.

Major objectives of the research are to:

1. Determine the effects of long-term, low level intakes of pesticides upon nutrient requirements of people.
2. Determine whether proper diet may protect against adverse effects of environmental levels of pesticides in the diet.
3. Identify and measure the effects of the use of pesticides during production upon the nutritive value of foods.
4. Determine whether and how diet and food use recommendations need to be adjusted as the result of the use of pesticides under allowed procedures.

Progress - USDA and Cooperative Program

A. Fumigation and Nutrient Value of Wheat

Fumigation of wheat during storage had little effect on nutrient content of wheat or of the bread and rolls derived from the wheat. There was some accumulation of bromine (from methyl bromide fumigant) and of phosphine. Overall, time of storage had more influence than the fumigation and other treatments on the decrease in quality characteristics of flour and the quality of the finished doughs and baked products. Methyl bromide left the wheat in better condition as evidenced by less fragmentation of the grain, although stale odor and crumbly texture of the baked products were detected in this case. This work has been completed and the findings accepted for publication.

Bromide residues in fumigated wheat did not increase the detrimental effect on rat thyroids of a diet marginal in iodine. Thyroids of male rats fed diets containing stored unfumigated wheat and marginal in iodine content were enlarged (weight 37.3 mg) as were those of rats fed similar diets containing wheat fumigated 8 times with methyl bromide (33.8 mg). Thyroids of rats fed similar diets adequate in iodine weighed 22.8 and 25.8 mg. Reasons for finding enlarged thyroids in rats fed diets containing stored wheat as compared to normal thyroids in rats fed similar diets made of the same wheat unstored are not apparent.

B. Soil Pesticides and Nutrient Content of Peanuts

The use of lindane in soil treatment for nematodes in the production of peanuts has long been known to alter metabolism of the plant leading to off-flavors in the nuts and a USDA recommendation not to use lindane. The use of lindane and associated metabolic changes, however, had little or no significant effect on the levels of several nutrients including vitamins, amino acids, fatty acids, fat, nitrogen and protein in the raw or roasted nuts. These findings came from contract research at the Texas Agricultural Experiment Station at College Station.

C. Retention of Insecticide Residues During Depletion of Body Fat

Growth and development of young rats and health of adult rats based on gross pathological examination were not adversely affected by the daily consumption of a low level of a mixture of chlorinated hydrocarbons for an extended period of time. Pesticide accumulation in the brain and fat tissue was similar with respect to the kind of diet. Lard as the source of fat in the diet yielded the lowest residues in these organs. Liver, however, stored the lowest amounts of pesticide when the main dietary fat was from peanut butter. Alteration of the amount of body fat by dietary restriction has not resulted in any ill-effects due to pesticide accumulation. Weight reduction resulted in mobilization of some pesticides, and the persistent storage of other pesticides, depending on the diet. Pesticide losses from the liver mainly occurred when the chief source of dietary fat was animal, lard or suet. As body stores of fat were depleted pesticides essentially continued to be stored, at least in the perirenal and epididymal fat pads except for some mobilization in rats consuming peanut butter. Brain resisted changes in pesticide content except for increased DDT content. Dieldrin concentration in the liver remained unchanged regardless of diet since the decreased content paralleled the decrease in liver weight. In contrast DDT content of liver did not change in spite of decreased organ weight. Pesticide content of adipose tissue from restricted animals generally remained constant except with rats fed peanut butter.

D. Response to Chlorinated Hydrocarbon Insecticides When Dietary Protein Is From Rice

Weanlings of second generation mothers fed rice diets were extremely small, but survival has been good, and the animals appear healthy. At 30 weeks these rats are still growing, although their body weights are much less than the casein controls. Lysine and lysine plus threonine supplementation improved growth rates somewhat. Addition of DDT and malathion has had no effect on weanling weights, food intake, or growth in any of the dietary groups. These results are from contract research with the Wisconsin Alumni Research Foundation at Madison.

E. Herbicides and Provitamin A in Plants

Results indicate that many published values for provitamin A content of vegetables are too high because they do not take into account the lower provitamin A values of stereoisomers of carotenes. Part of this research has been prepared for publication and has been accepted by the J. Assoc. Off. Anal. Chem.

F. Pesticides and Nutritive Composition

Significant increases were found in protein contents of pea and sweet corn seeds following soil treatments with triazine herbicides. Foliar application of triazines to seedlings of pea, sweet corn and bush bean significantly increased seedling (or leaf) protein contents and the activities of nitrate reductase and several other enzymes. At the same time decreased starch and sugar contents were observed. The commercially employed soil fumigants Telone and Nemagon increased the carotene content of carrots and reduced respiration rates. These results are from contract research with Utah State University at Logan.

Commercial use of a soil fumigant can result in a positive effect on the nutritional value of a commodity. In this case, the effect was to increase the β -carotene level in carrots grown in soil treated with three different soil fumigants, 1, 3-dichloropropene, ethylenedibromide, and dibromochloropropane. These results are from contract research with the University of California at Los Angeles and Riverside.

Publications - USDA and Cooperative Program

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